Patent Claims

1. A Method for producing a channel-forming protein, found in gram-positive bacteria, wherein the channel-forming protein is obtained by

a) heterologous overexpression or

b) purification from mycobacteria, whereby the extraction temperature is higher than 50°C.

2. A Method according to one of the aforementioned claims, wherein the gram-positive bacterium is one that contains at least one mycolic acid.

3. A Method according to one of the aforementioned claims, wherein the bacterium is a mycobacterium, preferably *Mycobacterium smegmatis*.

4. A Method according to one of the aforementioned claims, wherein the channel forming protein is a porin.

5. A Method according to one of the aforementioned claims, wherein the porin is essentially chemically stable against organic solvents.

6. A Method according to claim 4, wherein the porin is essentially thermally stable up to a temperature of 80°C, preferably 100°C.

7. A Method according to one of the aforementioned claims, wherein the porin is the porin MspA, MspC, MspD, a fragment of one of these porins, a homologous protein from one of these porins or their fragments, or a protein taken from a sequence of one of these porins.

8. A Method according to one of the aforementioned claims, wherein the heterologous overexpression is realized in *E. coli* or mycobacteria .

9. A Method according to one of the aforementioned claims, wherein a gene encoding a channel-forming protein, preferably a porin, is used for the overexpression.

10. A Method according to one of the aforementioned claims, wherein an *mspA* gene according to sequence 1, an *mspC* gene according to sequence 6, or an *mspD* gene according to sequence 8 is used for overexpression.

11. A Method according to one of the aforementioned claims, wherein a mutant gene derived from the sequences 1, 6, or 8 is used for overexpression, in which the mutation is essentially so that the chemical and thermal stability, as well as the channel-like structure, correspond essentially with that of MspA, MspC or MspD.

12. A Method according to one of the aforementioned claims, wherein the mutation is essentially so that the codon usage of the *mspA*, *mspC* or *mspD* gene is adapted to that of highly expressed genes in *E. coli*.

13. A Method according to one of the aforementioned claims, wherein a mutated *mspA*-, *mspC*- or *mspD* gene is used for overexpression where the mutation is essentially so that the G+C content is reduced to less than 66%.

14. A Method according to one of the aforementioned claims, wherein the *synmspA* gene according to sequence 4, is used for overexpression.

15. A Method according to one of the aforementioned claims, wherein a suitable vector for overexpression in *E. coli*, containing the *synmspA* gene according to sequence 4, is used.

16. A Method according to one of the aforementioned claims, wherein the channel-forming proteins are produced from the cell wall from gram-positive bacteria using non-ionic or zwitterionic detergents.

17. A Method according to one of the aforementioned claims, wherein the detergents used come from the following list: isotridecylpoly(ethyleneglycolether)$_n$, alkylglucosides, especially octylglucoside, alkylmaltoside, especially dodecylmaltoside, alkylthioglucosides, especially octylthioglucoside, octyl-polyethylenoxide and lauryldimethylaminoxide (??).

18. A Method according to one of the aforementioned claims, wherein the extraction temperature is between 80 and 110°C, preferably between 90 and 100°C.

19. A Method according to one of the aforementioned claims, wherein the extraction time is 5 - 120 min, preferably 25 - 35 min.

20. A Method according to one of the aforementioned claims, wherein a buffer with an ionic strength above 50 mM NaCl or Na-phosphate is used.

21. A Method according to one of the aforementioned claims, wherein the channel-forming protein is purified by precipitation, particularly using acetone.

22. A Method according to one of the aforementioned claims, wherein the channel-forming protein is purified using ion-

exchange chromatography, particularly an anion-exchange chromatography.

23. A Method according to one of the aforementioned claims, wherein the channel-forming protein is purified using size-exclusion chromatography.

24. A Method according to one of the aforementioned claims, wherein one of the aforementioned claims, wherein the channel-forming protein, produced through heterologous overexpression by raising the local concentration, is renatured.

25. A Method according to claim 24, wherein raising of the local concentration is realized by electrophoretic enrichment, especially by means of a DC current, by precipitation or adsorption at a suitable surface, especially at a membrane.

26. Channel-forming protein from a gram-positive bacterium, produced according to a method according to one of the aforementioned claims.

27. Channel-forming protein from a gram-positive bacterium, wherein the channel-forming protein is a porin that is essentially chemically stable against organic solvents.

28. Channel-forming protein from a gram-positive bacterium, wherein the channel forming protein is a porin essentially stable up to a temperature of 80°C.

29. Channel-forming protein according to claim 28, wherein the channel-forming protein is a porin that is essentially thermally stable up to a temperature of 100°C.

30. Channel-forming protein from a gram-positive bacterium, wherein the channel-forming protein is the porin MspA, MspC,

MspD, a fragment of these porins, a protein homologous to these porins or their fragments, or a protein derived from a sequence of these porins.

31. Channel-forming protein according to claim 30, wherein the chemical and thermal stability, as well as the channel-like structure of the deduced protein, is essentially that of the proteins MspA, MspC oder MspD.

32. Gene, encoding a channel forming protein according to one of the claims 26 - 31.

33. Gene according to claim 32, wherein the gene is the *mspA* gene according to sequence 1.

34. Gene according to claim 32, wherein the gene is the *mspC* gene according to sequence 6.

35. Gene according to claim 32, wherein the gene is the *mspD* gene according to sequence 8.

36. Mutated *mspA* gene, *mspC* gene or *mspD* gene, wherein the mutation is essentially such that the codon usage of the *mspA*, *mspC* or *mspD* gene is adapted to that of highly expressed genes in *E. coli*.

37. Mutated *mspA* gene, *mspC* gene or *mspD* gene, in particular according to claim 36, in which the mutation essentially consists of reducing the G+C content to less than 66%.

38. Mutated *mspA* gene, *mspC* gene or *mspD* gene, in particular according to claim 36 or 37, derived from one of the sequences 1, 6, or 8, in which the mutation is such that the chemical and thermal stability, as well as the channel-like structure

of the protein is for all practical purposes that of MspA, MspC or MspD.

39. Mutated *mspA* gene according to claim 36 through 38, wherein the mutated gene is the *synmspA* gene according to sequence 4.

40. Plasmid vector pMN501.

41. Overexpression system, in which *E. coli* contains the plasmid vector pMN501.